Table S1. Primer sequences used to confirm transposon insertion of different STM mutants and to generate plasmid constructs for complementation as well as purification of CyaB

Gene/Application	Primer sequences (5/ to 3/)
cyaB (BB0723)	cyaBNF:AATTCATTTTCTTTTTAGGTATAAGC cyaBCR: TTATTTTTTACTTTGATTTGCC
*pts*G (BB0645)	ptsGF: ATGTTAAAGGGTTTTGAACAAGCTC ptsGR: TCAAGCCTCTAGCAATTCTTCTA
*fru*A1 (BB0408)	fruA-1F:ATTTTTTGAAAAAAGATCTTG fruA-1R:TCATTCAGATTCCTTTAATTTT
*fru*A2 (BB0629)	fruA-2F:ATGCAAAATTTATTTTCAAAAAAC fruA-2R:TTAATCGTTTATATTCTTGTATTCTT
*mal*X1 (BB0116)	malX-1F: TTTATTAAAATAATTAACTTTTCAAG malX-1R: TAATCTTCTTCTAATTCGCTAA
*mal*X2 (BBB029)	malX-2F:ATGTCAACATCATCAGCATC malX-2R:TTACATAAGCTTGTCGATTTCC
*chb*A (BBB05)	chbAF:ATGAATAAAAAAATATATAGCATAGA chbAR:CTATTTTTTTATTTCATTTATT
*chb*C (BBB04)	chbCF: ATGAATTTTCAAGATTTTATTGAAAC chbCR: CTATTCTTTTTCCTGCAAAAGTAG
*chb*B (BBB06)	chbBF:ATGCTGGTACAAAGAATTGAA chbBR:TTAAACTGAACTTTTATTGTTAAATG
hpr (BB0448) hprF:	ATGCAAGAAGTAGAAATTGAGAT hprR: TTAAAGCTCTTTTGAAAAA
Detection of complemented plasmid	PKFSS1NF: CCCAATACGCAAACCGCCTCTC PKFSS1NR: TTATTTGCCGACTACCTTGGTG
ptsG complementation[a]	BamH1ptsGN: GGGGGATCCCAGACAATGATTTTAATTTTT SalIptsGC:GGGGTCGACAGCTATTACTTATTAAAAAAATCAAG
cyaB complementation[a]	cyaBFBamH1N2: GGGGGATCCATTCATTTTCTTTTTAGGTATAAGC cyaBRSalI: GGGGTCGACTTATTTTTTACTTTGATTTGCC
Recombinant CyaB expression[a]	*cyaB*FBamHIN:GGGGGATCCTTTGAAATAGAATCAAAAGCAT *cyaB*RSalIC: GGGGTCGACTTATTTTTTACTTTGATTTGCC

[a] underline indicates restriction site

Table S2. Primer sequences used for qRT-PCR and qPCR analysis

Gene	Primer sequences (5′ to 3′)
rpoS	rpoSF:CAGCAGCTCTTATTAATC rpoSR:CACGAAGAAGAAATCAAA
chbC	chbCF:CTACCTATAGCCTTTAGA chbCR:TGCTCTTCTTAATCCTATA
hpr	BB_0448F: GCACGAAGAATGTTTATTTG BB_0448R: GTACCAATGTTATTATTGC
vlsE	BB_F0041vlsE1F: GCGATATAAGTAGTACGA BB_F0041vlsE1R: CTGTCTTTACAGCTTTTAC
BB_0844	BB0844F: TCCACTATTTAATTGAGTA BB0844R: GAGAGAAATTAAGAATAAGA
dbpB	BBA25F: AAGCCTATTATTCCAACG BBA25R: GGACTAGCCTTAAGAGAA
dbpA	BBA 24F: TCCAGTTTCTTTGAGTTTA BBA 24R: CGCTAAAGACATTACAGA
ospC	BB_B19F: CTTGGTAAAGAAGGTGTTA BB_B20R: GCTCTTTAACTGAATTAGC
flaB	BB_0147flaBF: GCAGATTGTGTTAAAATACTA BB_0147flaBR: TCCTGTTAATGTTACAACTA
manA	BB407manAF: TGACCAACTCTTAACATC BB407manAR: CATGCATATCTTAAGGGA
fruA1	BB408fruA1F: CTCTCTTACCTTCAAATC BB0408fruA1R: CAAGGATCTATTGGTATTG
osm28	BBA74osm28F: TGCAGATTCTAACAATGC BBA74osm28R: CGGTTACCTTGTTAATAGTA
rpoN	BB_0450F:GATCGTAAGGGTTTATTATATG BB_0450R: CTGCAATTAAGAATTCAAAGA
plzA	BB0733plzAF: CTTCGCAATACTATTGAGA BB0733plzAF: GCACTACTCTTAGAATCTG
enolase	eno-BB0337F-RT: GCTTGAACTTGATGGCACCCCTAC eno-BB0337R-RT: GTACGCTCCAAGATATTGATAAGG
flaB (qPCR)	De FlaBF-GGAGCAAACCAAGATGAAGC De FlaBR-AGCTCCCTCACCAGAGAAAAG
β-actin (qPCR)	JS b-Actin-F : ACGCAGAGGGAAATCGTGCGTGAC JS b-Actin-R : ACGCGGGAGGAAGAGGATGCGGCAGTG

Supplemental Figure Legends

Fig. S1. PCR assay to verify the insertion site and detect the presence of co-isolated clones in different PEP-PTS and *cyaB* transposon mutants generated in a prior study (1). Primers (Table S1) were designed that flanked the previously determined insertion site, and each set was used for PCR amplification from the 5A18NP1 parental strain (A) and the transposon mutant (B). Presence of the transposon insertion resulted in an increase in size of 1797 bp, whereas a band the same size as that found with 5A18NP1 indicated in the presence of co-isolated clones with different insertion sites. White arrows indicate contamination of the mutant clone with other transposon mutant(s) that have insertions at other sites. Blue arrows indicate mutant that the isolate did not contain a transposon insertion at the expected site.

Fig. S2. *B. burgdorferi* burdens in skin and mouse tissues of different PEP-PTS mutants. *flaB* copy numbers were detected by qPCR, representing the number of *B. burgdorferi* genomes. β-actin copy numbers were also determined in these tissues to normalize the *flaB* copy to the host tissue DNA. Each data point in the graph represents the number of copies of *Bb* genomes per 10^6 copies of β-actin. The geometric mean $\pm$ SEM of the three biological replicates are shown. The Student's t-test was not significant ($p>0.05$) for both skin and heart samples when the data for individual mutants were compared with WT.

Fig. S3. Parental 5A18NP1, *ptsG* (BB0645) and *fruA2* (BB0629) (T11P02A09) mutants of *B. burgdorferi* showed similar growth kinetics in BSKII and BSK basal medium containing low levels of glucose (BSK-Lite). Midlog cultures of each strain were inoculated into 6 ml of fresh BSKII medium and BSK-Lite (10^6/ml) in triplicate, and bacteria were counted each day for 9 days using dark-field microscopy. To remove residual glucose in the BSK-Lite medium studies, the starter cultures for each strain were centrifuged and re-suspended into fresh BSK-Lite medium prior to inoculation. Each data point in the graph indicates the mean $\pm$ S.E. of three biological replicates. One-way ANOVA analysis (Kruskal-Wallis test) showed that the growth of the three strains was not significantly different ($p>0.05$) from one another in either medium condition.

Fig. S4. PCR assay to determine shuttle vector of *ptsG* complemented strain (*ptsG*:: PKFSS-1-*ptsG*) in BSKII medium after 5 passages of growth in the presence and absence of antibiotic selection. Lane A: each passage without streptomycin; Lane B: each passage with streptomycin;

shuttle vector possesses a streptomycin cassette. *ptsG* complemented strain lost the shuttle plasmid after 4 and 5 passages of growth in the absence of streptomycin whereas the same strain retained the shuttle plasmid when cultured in the presence of streptomycin.

Fig. S5. Reverse transcriptase (RT) PCR to identify the transcripts of wild type (WT), *cyaB* mutants and complemented strains of *B. burgdorferi*. Results showed that WT and *cyaB* complemented strains produced *cyaB* transcript whereas none of the *cyaB* mutant, transposon insertion either in the 5′(*cyaB*N) or 3′ site (*cyaB*C), does not. Lanes: 1, DNA ladder; 2, WT-with RT; 3, WT-without RT; 4, *cyaB*N-with RT; 5, *cyaB*N-without RT; 6, *cyaB*C-with RT; 7, *cyaBC*-without RT; 8, *cyaB* complemented-with RT; 9, *cyaB* complemented-without RT.

Fig. S1

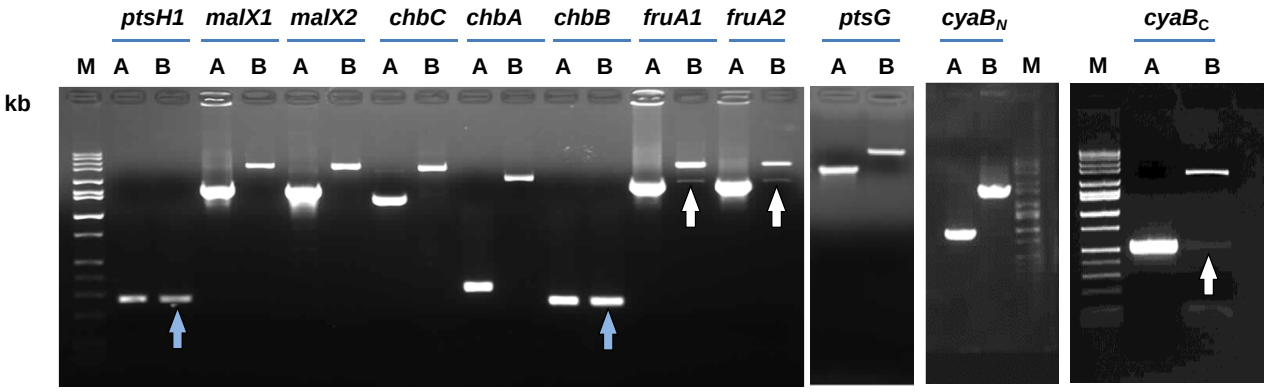

ptsH1
malX1
malX2
chbC
chbA
chbB
fruA1
fruA2
ptsG
cyaB$_N$
cyaB$_C$
M A B A B A B A B A B A B A B A B A B A B M M A B
kb

Fig. S2

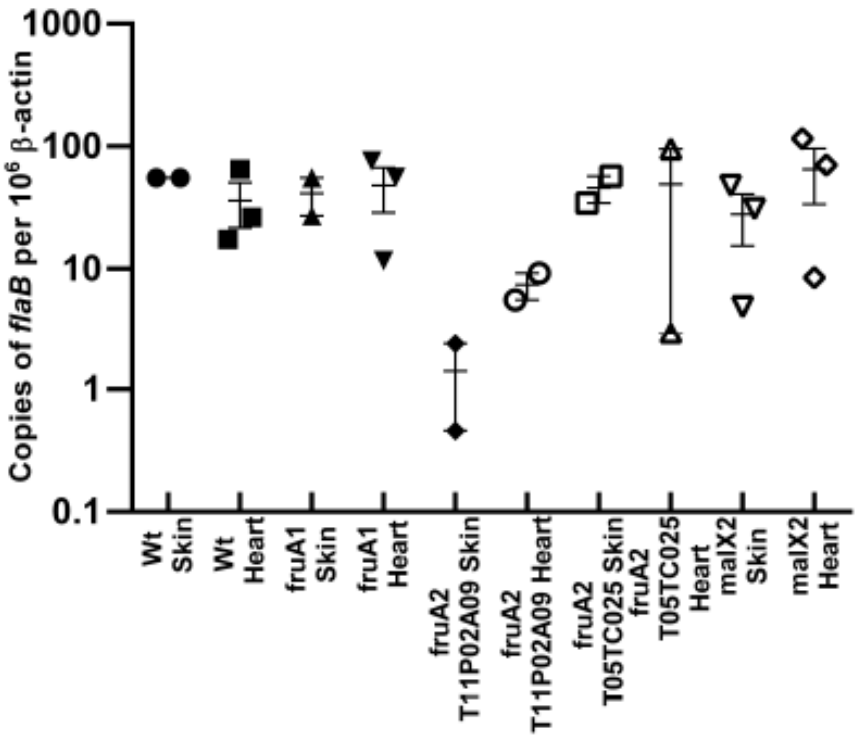

Copies of flaB per 10^6 β-actin
1000
100
10
1
0.1
Wt Skin
Wt Heart
fruA1 Skin
fruA1 Heart
fruA2 T11P02A09 Skin
fruA2 T11P02A09 Heart
fruA2 T05TC025 Skin
fruA2 T05TC025 Heart
malX2 Skin
malX2 Heart

Fig. S3

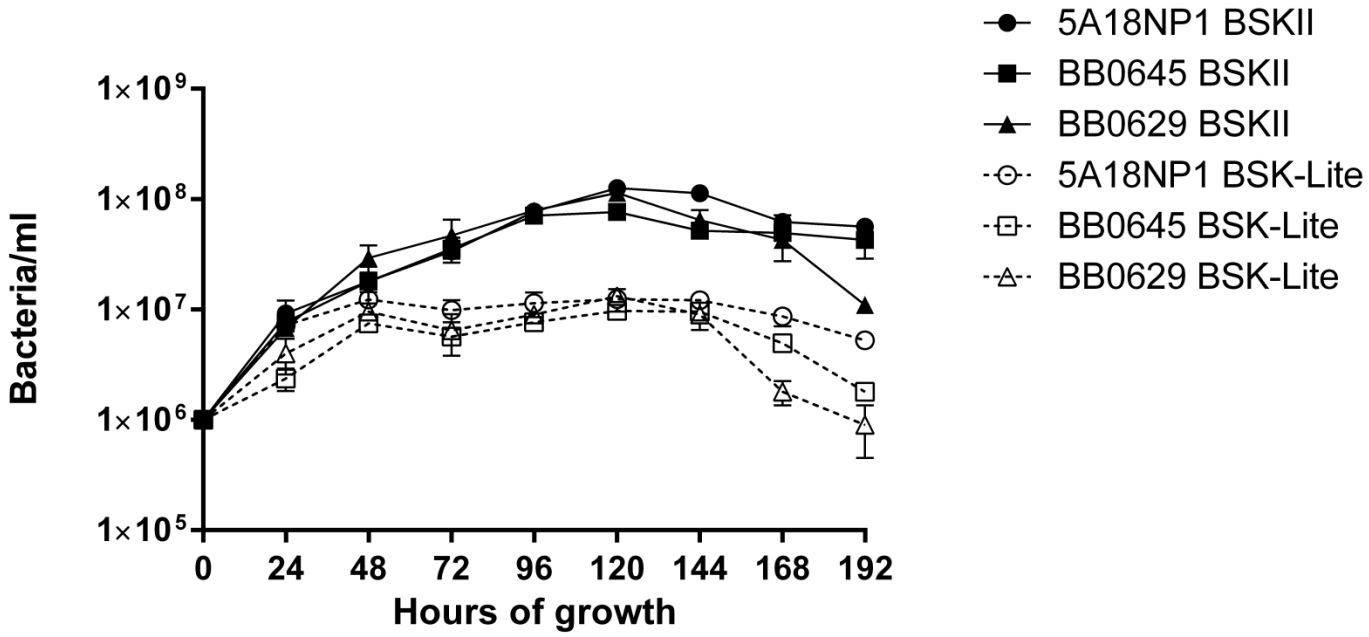

Bacteria/ml
1×10⁹
1×10⁸
1×10⁷
1×10⁶
1×10⁵
0
24
48
72
96
120
144
168
192
Hours of growth
5A18NP1 BSKII
BB0645 BSKII
BB0629 BSKII
5A18NP1 BSK-Lite
BB0645 BSK-Lite
BB0629 BSK-Lite

Fig.S4

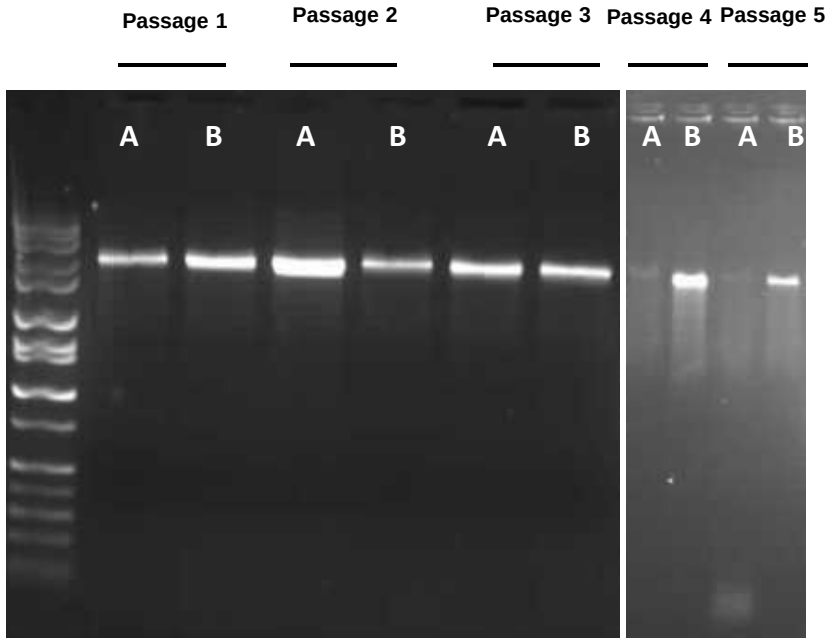
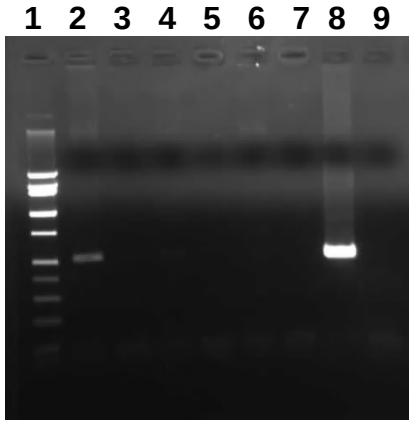

Passage 1
Passage 2
Passage 3
Passage 4
Passage 5
A
B
A
B
A
B
A
B
A
B

Fig. S5



Reference:

1. **Lin T, Gao L, Zhang C, Odeh E, Jacobs MB, Coutte L, Chaconas G, Philipp MT, Norris SJ.** 2013. Analysis of an ordered, comprehensive STM mutant library in infectious *Borrelia burgdorferi*: insights into the genes required for mouse infectivity. PLoS One **7:**e47532.